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# **Enzyme Chemical Engineering and Its Application to Biosensors**

**A thesis presented in partial fulfilment of the requirements  
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## ABSTRACT

Enzyme chemical engineering is a fast growing area in biotechnology. It has been used to change the stability, solubility, activity and other properties of enzymes for more control over and wider application of enzymes.

In this thesis, this technology is applied to another new and fast growing area of research: biosensors. Over the last decade, biosensors are gaining increasing awareness as a highly attractive analytical tool. One of the current challenges in this area is to identify a universal and scaleable way to produce sensitive, stable, instantaneous, and easy to prepare biosensors for mass production

In this study, enzyme chemical engineering is adopted as a new approach and glucose oxidase is served as a model to build a biosensor system in attempting to address the above challenge.

In the study, glucose oxidase was used as the catalyst to chemically amplify the redox reaction of glucose. Haemin was employed as the bifunctional promoter to act as a "bridge" to connect glucose oxidase (GOD) and electrode. Haemin, similar to ferrocene also acts as a mediator to transfer electrons between the active center of the enzyme and the electrode.

In the construction of a haemin-glucose oxidase biosensor, haemin was covalently bound with glucose oxidase. The haemin-glucose oxidase conjugate was then chemisorbed on to the platinum electrode to modify the electrode surface and form an "enzymatic redox center-bridge-electrode" system. The modification of the glucose oxidase with haemin comprised of two steps: converting the haemin carboxyl group to the reactive enol ester and then covalently bonding to an amino group of glucose oxidase. For chemisorption, the electrode was soaked in a solution of the haemin-glucose oxidase conjugate in phosphate buffer solution (pH 7.0) at 4°C for 16 hours. The same experiment was carried out by using unmodified glucose oxidase as a blank.

The following facts proved that the covalently bound haemin-glucose oxidase system was formed successfully: 1) The large molecule fractions eluted from the Sephadex G-10 gel column had the enzyme activity and other characteristics of glucose oxidase. 2) The same fractions retained about 2/3 to 3/4 of the specific activity of original glucose oxidase. 3) The absorbance spectra of these fractions showed the peaks corresponding to both haemin and glucose oxidase.

The following evidence suggests that the haemin-glucose oxidase conjugate was successfully chemisorbed on to the electrode surface: 1) The cyclic voltammogram of the electrode chemisorbed with conjugate was completely different from that adsorbed with glucose oxidase alone. 2) The cyclic voltammogram of the conjugate chemisorbed electrode in the solution with glucose was quite distinct from that without glucose. Thus a different species from either glucose oxidase or haemin was chemisorbed on to the electrode.

Furthermore, the conjugate chemisorbed electrode showed linearity between current response and glucose concentration at a range from 0mM to 10mM. The ratio of the current response to glucose concentration was about 1.6 $\mu$ A/mM. However, the platinum electrode adsorbed by GOD alone had a poor response to glucose. The response time of the system of platinum electrode-haemin-glucose oxidase was very rapid at less than one minute, and the response fell initially but then remained stable over a period of 14 days.

Thus the experimental data proved that the system of platinum electrode-haemin-glucose oxidase met the requirements for a glucose sensor in the factors of the sensitivity, linear response range, lifetime, ease of preparation, convenience of operation, non-toxicity and low cost. In other words, it demonstrated the characteristics of a glucose biosensor.

Finally, using the preparation of this glucose biosensor as a model, the electrochemical mechanism of the biosensor system was proposed. The model was also used to suggest a systematic approach for constructing amperometric biosensors. The extension of this approach and the potential applications of

this type of biosensor are also discussed.

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## TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS	v
LIST OF FIGURES	ix
LIST OF TABLES	xi
LIST OF ABBREVIATIONS	xii
CHAPTER ONE INTRODUCTION	1
1.1 Biosensors.....	1
1.2 Amperometric biosensor.....	1
1.3 Glucose oxidase (GOD) sensors .....	3
1.3.1 Direct detection method.....	4
1.3.2 Indirect detection involving a mediator.....	7
1.3.3 Modification of electrode.....	9
1.4 Aim and content of this thesis .....	10
CHAPTER TWO METHODS AND EQUIPMENT	12
2.1 Haemin assay .....	12
2.1.1 Haemin assay by atomic absorption spectrometry .....	12
2.1.2 Haemin assay by UV/Vis spectrometer .....	13
2.2 Glucose oxidase concentration assay .....	14
2.2.1 Instrument and Reagent .....	14
2.2.2 Procedures .....	14
2.3 Assaying the activity of glucose oxidase.....	15
2.3.1 Instrument and reagents.....	15
2.3.2 Procedures .....	15
2.4 Conjugating haemin to glucose oxidase .....	15
2.4.1 Reagent.....	16

2.4.2	Procedure .....	16
2.5	Separation of haemin –GOD conjugate from free haemin .....	17
2.5.1	Reagent .....	17
2.5.2	Procedure .....	17
2.6	Electrode preparation .....	18
2.6.1	Preparation of platinum wire auxiliary electrode .....	18
2.6.2	Preparation of reference electrode .....	18
2.6.3	Preparation platinum disc electrode.....	19
2.6.4	Preparation of working electrode .....	19
2.6.5	Cyclic voltammetry assay .....	20

### CHAPTER THREE RESULTS AND DISCUSSION 21

3.1	Structure and properties of glucose oxidase and haemin .....	21
3.1.1	Structure and properties of glucose oxidase .....	21
3.1.2	Structure and properties of haemin.....	24
3.2	Assay haemin .....	26
3.2.1	Solvent system .....	26
3.2.2	Assay haemin by determination of ferric content in haemin.....	26
3.2.3	Assay haemin by UV/Vis spectrometer.....	28
3.3	Assay concentration of glucose oxidase.....	32
3.3.1	Principle of the assay concentration of glucose oxidase.....	32
3.3.2	Calibration graph for concentration of glucose oxidase .....	32
3.4	Activity calibration of glucose oxidase .....	33
3.5	Detection of haemin-GOD conjugate .....	34
3.5.1	Concentration of GOD in the conjugate .....	34
3.5.2	GOD activity in haemin-GOD conjugate .....	35
3.5.3	Specific activity of haemin-GOD conjugated.....	36
3.5.4	Comparison of the activity of haemin-GOD conjugate with that of GOD found.....	37
3.5.5	Absorbance spectra of the purified haemin-GOD conjugate.....	42
3.6	Electrochemical detection of platinum electrode chemisorbed with the haemin-GOD conjugate .....	43
3.6.1	Cyclic voltammetry.....	43
3.6.2	Cyclic voltammetry assay .....	44



3.7	Electrochemical measurement.....	48
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CHAPTER FOUR CONCLUSION	54
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4.1	Finding optimum conditions for the conjugation and the chemisorption .....	54
4.2	Studying the effects of the modifying the enzyme and the electrode ..	58
4.3	Evolving an approach for the construction an amperometric biosensor .....	62
4.4	Identifying areas for further study .....	63
4.5	Discussion of the current and future applications of this type of biosensor .....	65

REFERENCES	68
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## LIST OF FIGURES

Figure 1.1	A comparison of the relative merits of the various transducer technologies used in the construction of biosensors .....	3
Figure 1.2	Amperometric enzyme electrode based on a Radiometer E5046 Clark oxygen sensor.....	5
Figure 1.3	The structure of ferrocene .....	8
Figure 3.1	Representation of the overall folding of glucose oxidase.....	22
Figure 3.2	Structure formula of ferrihaemins .....	24
Figure 3.3	Calibration graph for ferric concentration in haemin comparison of two determination.....	27
Figure 3.4	Absorbance spectra for haemin and glucose oxidase .....	29
Figure 3.5	Comparison graph for haemin with and without sodium dithionite .....	30
Figure 3.6	Absorbance spectra for oxidized (A) and reduced (B) haemin species in the buffer solution (pH 7.0) .....	31
Figure 3.7	Calibration graph for haemin .....	32
Figure 3.8	Calibration graph for concentration of glucose oxidase (GOD) .....	33
Figure 3.9	Calibration graph for GOD activity at varied concentrations of GOD ( $\mu\text{g/ml}$ ) .....	33
Figure 3.10	Calibration graph for GOD activity at varied determination time (min).....	34
Figure 3.11	Activity graph for haemin-GOD conjugate .....	35
Figure 3.12	GOD activity curves for haemin-GOD conjugate and GOD found .....	38
Figure 3.13	Stored effect graph for the GOD activity of GOD found at 4°C in the buffer pH 7.0 .....	38
Figure 3.14	Stored effect graph for the GOD activity of haemin-GOD conjugate at 4°C in the buffer pH 7.0.....	39
Figure 3.15	Stability of GOD activity in GOD found stored at 4 °C in buffer pH 7.0.....	40
Figure 3.16	Stability graph for GOD activity of haemin-GOD conjugate stored at 4 °C in buffer pH 7.0.....	40

Figure 3.17	Comparison the GOD specific activity of haemin-GOD conjugate with that of GOD found.....	41
Figure 3.18	Absorbance spectra for GOD, haemin and conjugate .....	42
Figure 3.19	Cyclic voltammograms .....	45
Figure 3.20	Cyclic voltammograms for the platinum electrode chemisorbed with the haemin-GOD conjugate. ....	46
Figure 3.21	Cyclic voltammogram for TTF modified glucose oxidase at a platinum-disc electrode.....	48
Figure 3.22	Current responses to glucose.....	49
Figure 3.23	Calibration graph for measurement of glucose at platinum electrode chemisorbed by haemin-GOD conjugate .....	50
Figure 3.24	Stability of response for electrode chemisorbed by haemin-GOD conjugate in 5mM glucose of buffer solution pH 7.0 .....	51
Figure 3.25	Calibration graph for glucose obtained with a ferrocene-modified glucose entrapped within a polypyrrole film .....	51
Figure 4.1	Entrapment of mediator-modified enzyme within a conducting-polymer film .....	60
Figure 4.2	Haemin conjugate the glucose oxidase and the electrode surface.....	61
Figure 4.3	The general features and some examples of the biofunctional molecules. ....	64

## LIST OF TABLES

Table 3.1	Comparison of the calculated value with the determined value of iron and the calculated value with added value of haemin ..	28
Table 3.2	Concentration of GOD in the fractions .....	35
Table 4.1	Some flavoproteins and their applications.....	63

## LIST OF ABBREVIATIONS

DCC	Dicyclohexycarbondi-imide
DEC	1-(3-dimethylaminopropyl)-3-ethylcarbodi-imide hydrochloride
EFGP	Electrode-ferrocene-GOD entrapped within polypyrrole film
EHG	Electrode-haemin-GOD
FAD	Flavin adenine dinucleotide
FADH <sub>2</sub>	Reduced flavin adenine dinucleotide
GOD	Glucose oxidase
HGOD	Conjugated haemin-glucose oxidase
HOSu	Hydroxysuccinimide
Mox	The oxidised form of mediator
Mred	The reduced form of mediator
Na-HEPES	Sodium 4-(2-hydroxyethyl)-1-piperazine-ethane-sulfonate
PDR	Protein determination reagent
POD	Horseradish Type II peroxidase
SGE	"Sandwiched" glucose oxidase electrode
TTF	Tetrathiafulvalene
UV	Ultraviolet
Vis	Visible